EXECUTIVE SUMMARY
to
Application to Food Standards Australia New Zealand for the Inclusion of Maize MON 94804 in *Standard 1.5.2 - Food Produced using Gene Technology*

Submitted by:

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EXECUTIVE SUMMARY

Food/Feed Safety and Nutritional Assessment of MON 94804 Maize

Bayer has developed short stature maize MON 94804 that contains a suppression cassette expressing an inverted repeat sequence designed to target endogenous maize *gibberellic acid 20 oxidase* (*GA20ox*) genes, *ZmGA20ox3* and *ZmGA20ox5*. The expressed inverted repeat transcript is recognized by the endogenous RNA interference (RNAi) machinery, resulting in down-regulation of the targeted *GA20ox* gene expression. This suppression results in the reduction of gibberellic acid/gibberellin (GA) levels in the stalk, leading to a reduction of internode length and consequently reduced overall plant height compared to the conventional control maize.

MON 94804 maize can provide agronomic and environmental benefits, including reduced lodging and green snap, season-long crop access using standard ground equipment, and potential for improved environmental sustainability with more precise, well-timed, and "as needed" mid to late -season application of agrochemicals (*e.g.*, fungicide) and/or key nutrients (*e.g.*, nitrogen). This product is intended for cultivation in North America, South America, and potentially other key maize markets.

MON 94804 maize will be combined with other authorized biotechnology-derived traits through traditional breeding methods to provide growers with products that offer protection against maize pests, herbicide tolerance, other traits offering broader grower choice, and the potential for improved yield protection.

Molecular Characterization of MON 94804 Maize Verifies the Integrity and Stability of the Inserted DNA

MON 94804 maize was produced by *Agrobacterium tumefaciens*-mediated transformation of maize tissue using the transfer DNA (T-DNA) transformation vector PV-ZMAP527892. This vector contains a single T-DNA, that is delineated by Right and Left Border regions and the final T-DNA contains the *GA20ox_SUP* suppression cassette. The T-DNA that was initially inserted contained a *cp4 epsps* selectable marker cassette flanked by *loxP* sites. After a successful transformant was selected, the selectable marker cassette was excised by crossing the transformant with a Cre recombinase expressing line (the Cre line was transformed with the vector PV-ZMOO513642). A selectable marker-excised line was selected as MON 94804 maize. Subsequently, segregation, selection and screening were used to isolate those plants that contained the *GA20ox_SUP* suppression cassette and lacked plasmid vector backbone and the *cp4 epsps* selectable marker sequences from PV-ZMAP527892 and any sequences from the *cre* gene-containing vector, PV-ZMOO513642.

Characterization of the DNA insert in MON 94804 maize was conducted using a combination of sequencing, polymerase chain reaction (PCR), and bioinformatic analyses. The results of this characterization demonstrate that MON 94804 maize contains one copy of the intended T-DNA containing the *GA20ox_SUP* suppression cassette that is stably inherited over multiple generations and segregates according to Mendelian principles of inheritance. These conclusions are based on several lines of evidence:

- Molecular characterization of MON 94804 maize by Next Generation Sequencing (NGS) demonstrated that MON 94804 maize contained a single intended T-DNA insert. These whole genome sequence analyses provided a comprehensive assessment

of MON 94804 maize to determine the presence and identity of sequences derived from PV-ZMAP527892 and demonstrated that MON 94804 maize contained a single T-DNA insert and no plasmid backbone or *cp4 epsps* selectable marker sequence from PV-ZMAP527892 or any sequences from PV-ZMOO513642.

- Directed sequencing (locus specific PCR, DNA sequencing and analyses) performed on MON 94804 maize was used to determine the complete sequence of the single T-DNA insert from PV-ZMAP527892, the adjacent flanking genomic DNA, and the 5' and 3' insert-to-flank junctions. This analysis confirmed that the sequence and organization of the inserted T-DNA is identical to the corresponding region in the PV-ZMAP527892 T-DNA and lacks the *cp4 epsps* selectable marker.

- Furthermore, the genomic organization at the insertion site in MON 94804 maize was assessed by comparing the sequences flanking the T-DNA insert in MON 94804 maize to the sequence of the insertion site in conventional maize. This analysis determined that no major DNA rearrangement occurred at the insertion site in MON 94804 maize upon DNA integration, although there was a 41 bp deletion that likely occurred upon T-DNA integration in MON 94804 maize.

- Generational stability analysis by NGS demonstrated that the single PVZMAP527892 T-DNA insert in MON 94804 maize has been maintained through five breeding generations, thereby confirming the stability of the T-DNA in MON 94804 maize.

- Segregation analysis corroborates the insert stability demonstrated by NGS and independently establishes the nature of the T-DNA as a single chromosomal locus that shows an expected pattern of inheritance.

Taken together, the characterization of the genetic modification in MON 94804 maize demonstrates that a single copy of the intended T--DNA was stably integrated at a single locus of the maize genome and that no PV-ZMAP527892 plasmid vector backbone, *cp4 epsps* selectable marker, or PV-ZMOO513642 sequences are present in MON 94804 maize.

GA20ox_SUP miRNA is Safe for Consumption in Food or Feed

There is no evidence to suggest that dietary consumption of nucleic acids is associated with toxicity (Petrick *et al.*, 2013; U.S. FDA, 1992). U.S. Environmental Protection Agency (EPA) has an established tolerance exemption for nucleic acids that encode for plant-incorporated protectant (PIP) products (U.S. EPA, 2001). U.S. Food and Drug Administration (FDA) recognizes that all food allergens are proteins (U.S. FDA, 1992) and there is also no evidence of allergenicity of dietary RNA in the peer-reviewed scientific literature. This lack of toxicity or allergenicity for ingested RNA also extends to RNA molecules associated with RNAi-mediated gene regulation. Therefore, an extensive history of safe consumption of dietary RNAs, including double-stranded RNA (dsRNA), small interfering RNA (siRNA), and microRNA (miRNA), has been established as reviewed in Petrick *et al.* (2013). One of the reasons for this history of safe consumption of dietary RNAs is that extensive sequence-independent physiological and biochemical barriers are known to exist in humans and other animals that limit the potential for uptake or activity of ingested nucleic acids (Juliano *et al.*, 2009; O'Neill *et al.*, 2011; Petrick *et al.*, 2013; Rodrigues and Petrick, 2020). Additionally, regulatory agencies have concluded that ingestion of RNA molecules does not present a hazard to humans or other mammals.

GA20ox_SUP RNA produced in MON 94804 maize is processed to a miRNA that causes gene suppression of the targeted ZmGA20ox genes within the maize plant (Paciorek *et al.*, 2022). An extensive literature search indicates no evidence for the presence of GA biosynthetic pathway or GA20ox genes in humans or animals (Keswani *et al.*, 2022; Salazar-Cerezo *et al.*, 2018). The ubiquitous nature of gene suppression utilizing miRNAs in a wide variety of extensively consumed plant species, the long history of safe consumption of RNA molecules including miRNAs from a range of sources, and the apparent lack of toxicity or allergenicity of dietary RNAs including miRNA, it can be concluded that the GA20ox_SUP miRNA produced in MON 94804 maize poses negligible risks to humans or animals. Therefore, the GA20ox_SUP miRNA from MON 94804 maize or its progeny is considered safe for humans and animals.

Compositional Analysis of MON 94804 Maize Demonstrate Equivalence to the Conventional Maize

Safety assessments of biotechnology-derived crops follow the comparative safety assessment process in which the composition of grain and/or other raw agricultural commodities of the biotechnology-derived crop are compared to the appropriate conventional control that has a history of safe use.

Compositional analyses were conducted on grain and forage of MON 94804 maize and the conventional control grown at five sites in the U.S. during the 2020 field season. The compositional analysis provided a comprehensive comparative assessment of the levels of key nutrients, anti-nutrients and secondary metabolites in grain and forage of MON 94804 maize and the conventional control. The analyses followed considerations relevant to the compositional quality of maize as defined by the Organization for Economic Co-operation and Development (OECD) consensus document (OECD, 2002). Grain samples were analyzed for moisture and levels of key nutrients including proximates, carbohydrates by calculation, fiber, amino acids, fatty acids, minerals, and vitamins. In addition, grain samples were analyzed for levels of the anti-nutrients phytic acid and raffinose and secondary metabolites ferulic acid, furfural and p-coumaric acid. Forage samples were analyzed for moisture and levels of proximates, carbohydrates by calculation, fiber, and minerals. In total, 78 different components were analyzed (69 components in grain and 9 components in forage).

The results of the compositional assessment found that there were no compositional differences that were biologically meaningful between MON 94804 maize and conventional control maize and support the conclusion that MON 94804 maize is compositionally equivalent to conventional maize. These results support the overall food and feed safety of MON 94804 maize.

Conclusion

The data and information presented in this safety summary provide a weight of evidence that supports the conclusion that the food and feed derived from MON 94804 maize and its progeny are as safe and nutritious as food and feed derived from conventional maize. The food and feed safety of MON 94804 maize is based on the following lines of evidence:

1. A detailed molecular characterization of the inserted DNA demonstrated a single, intact copy of the expected T-DNA insert at a single locus within the MON 94804 maize genome and the absence of plasmid vector backbone and *cp4*

epsps selectable marker sequence. The genetic elements are present in the expected order and are inherited following Mendelian principles of inheritance.

2. Extensive literature search on safety of nucleic acids including miRNAs (*e.g.*, GA20ox_SUP miRNA) supports that it does not pose any meaningful risk to food safety. Also, according to the U.S. FDA (1992), nucleic acids, which are present in the cells of every living organism, do not raise concerns as a component of food, and are GRAS.

3. The comprehensive compositional assessment demonstrated that MON 94804 grain and forage are compositionally equivalent to grain and forage from conventional maize.

Therefore, the data herein demonstrate that the food derived from MON 94804 maize and its progeny are as safe and nutritious as food derived from conventional maize.

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